EXPERIMENTAL CROSSING OF *AEDES* (*STEGOMYIA*) *AEGYPTI* LINNAEUS AND *AEDES* (*STEGOMYIA*) *ALBOPICTUS* SKUSE (DIPTERA, CULICIDAE).

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(Plate xiv.)

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Synopsis.

Experimental crossings were made between various strains of *A. aegypti* and *A. albopictus*. Out of a total of 49,649 eggs deposited all were sterile, with the exception of one egg which gave rise to a male adult which showed thoracic markings combining the characters of the parent species.

Introduction.

This cross was first attempted by Toumanoff in 1937 and since then by various workers; the results of these experiments have been fully discussed by Kitzmiller (1953) and by Mattingly (1956) and a complete list of references will be found in their papers. The results obtained by various authors may be briefly summarized as follows: in all cases where fertile progeny was obtained the reciprocal cross was sterile, while the F_1 progeny and succeeding generations all completely resembled the female parent with the exception of one female in Toumanoff's and one male from a back cross in Bonnet's experiments which resembled the male parent. In some crosses the fertile progeny were produced by using *A. aegypti* as the female parent and in others by using *A. albopictus* as the female. Other workers again found complete sterility in both reciprocal crosses. In view of these contradictory results the author carried out a series of experiments during the period 1955–1959, the results of which are presented in this paper.

Methods.

Standard laboratory colonies of various strains of *A. aegypti* and *A. albopictus* were maintained and eggs from these were used to obtain adults for the crossing experiments. Single pupae were isolated in tubes and the resulting adults were checked twice for species and sex before being liberated in the cages. Mass matings were made, with the numbers of males and females per cage varying from approximately 100 to 250. In all experiments examinations were made of spermathecae of small samples of females for the presence of living spermatozoa. Eggs from the matings were deposited on rough textured white filter papers placed in small dishes of water, and these were kept wet for 48 hours to allow the embryos to develop and were then dried out slowly and kept at 80°F. and 90% relative humidity for 7 days before being counted and immersed in water; they were then kept in water to observe hatching for at least 10 days before being discarded. Small quantities of dried yeast and ground rat biscuits were added to the water, as it has been suggested that some organic pollution stimulates hatching, although this has not been the author's experience. The adults were kept in nylon marquisette cages 12″ × 10″ × 10″, were given raisins, sugar solution and fruit, and offered blood feeds three times weekly. The breeding work was carried out in a warm room at 80°F. and 75% to 80% relative humidity with natural daylight, including some hours of direct sunlight.

Eggs from the standard colonies treated as described above were 100% fertile. After the eggs from any particular culture or cross were handled, all glassware, instruments and bench surfaces were sterilized by heat treatment and the hands of the author thoroughly washed in very hot water. All cages were sterilized by dry heat before being used. These methods were also applied at all times to the handling of standard colonies.

The work was carried out entirely by the author with the exception of counting the eggs, which was carried out by an assistant who maintained the same precautions.

Results.

During the period 1955–1957 crosses were attempted between *A. aegypti* from Mornington Island, Queensland (Q.), and two strains of *A. albopictus*, one from the Philippines (P.) and the other from Singapore (S.). Specimens of the strain of *A. aegypti* used were submitted to P. F. Mattingly, who stated that they were mainly var. *queenslandensis* with a few dark enough to be considered as the type form (see Mattingly, 1957).

The details of the experiments are shown in Table 1.

Table 1.

Experiment Number.	Date.	Number of Mosquitoes, Sex and Species.	Number of Blood Feeds.	Number of Eggs Deposited.	Number of Eggs Hatched.	♀♀ Examined for Living Sperms.	
						Number Positive.	Number Negative.
1	June, 1955	254♀ alb. (P) × 245♂ aeg. (Q).	370	177	0	3	22
		250♀ aeg. (Q) × 260♂ alb. (P).	660	6,538	0	12	13
2	June, 1956	122♀ alb. (S) × 133♂ aeg. (Q).	210	1,676	1	4	6
		100♀ aeg. (Q) × 105♂ alb. (S).	220	349	0	1	9
3	July, 1957	182♀ alb. (P) × 241♂ aeg. (Q).	285	1,565	0	1	9
		186♀ aeg. (Q) × 246♂ alb. (P).	560	1,089	0	0	10
4	Sept., 1957	180♀ alb. (S) × 216♂ aeg. (Q).	305	837	0	1	19
		180♀ aeg. (Q) × 243♂ alb. (S).	435	8,757	0	0	20
				20,988			

Copulation was observed frequently in all crosses and in most cases living spermatozoa were observed in at least some of the females. The females fed freely, but the number of eggs deposited did not always correspond with the number of blood feeds taken. The number of females examined for living spermatozoa was too small to allow of any correlation between these figures and the number of eggs deposited. It will be seen that out of a total of 20,988 eggs, only one was fertile. This was bred through to the adult stage and resulted in a male specimen which was completely intermediate in dorsal thoracic markings between *A. aegypti* and *A. albopictus* (see Plate xiv). The genitalia of this specimen were submitted to P. F. Mattingly, who considers that they resemble *A. albopictus* with some slight modification in the direction of *A. aegypti*.

The most recent report of successful crosses between *A. aegypti* and *A. albopictus* is that by Bonnet in Hawaii in 1950. It was thought that the failure to repeat the results of previous experiments may have been due to the presence of different strains of the species used. Accordingly eggs of both species were obtained from Hawaii (H.), and the results of a series of crosses made with these during 1958–1959 are shown in Table 2.

Table 2.

Experiment Number.	Date.	Number of Mosquitoes, Sex and Species.	Number of Blood Feeds.	Number of Eggs Deposited.	Number of Eggs Hatched.	♀♀ Examined for Living Sperms.	
						Number Positive.	Number Negative.
1	Oct., 1958	106♀ alb. (H) × 127♂ aeg. (H).	172	3,169	0	5	5
		128♀ aeg. (H) × 182♂ alb. (H).	432	13,326	0	3	7
2	Feb., 1959	143♀ alb. (H) × 188♂ aeg. (H).	151	3,227	0	12	8
		140♀ aeg. (H) × 194♂ alb. (H).	495	8,939	0	6	14
				28,661			

As before, frequent copulation was observed in all crosses and at least some of the females showed living spermatozoa in the spermathecae. In this series *A. aegypti* fed much more freely than *A. albopictus*, and this showed a distinct correlation with the number of eggs deposited. It will be seen that of a total of 28,661 eggs deposited all were completely sterile.

Discussion.

The experiments outlined above were carried out on a much larger scale than those made by previous workers and all possible precautions were taken to avoid contamination of cultures by stray eggs. Two different strains of *A. aegypti* were used and three different strains of *A. albopictus*, though not in all the possible combinations. It is surprising therefore that the results of previous workers could not be confirmed. This leads to consideration of the possibility that previous results may have been due to the contamination of cultures by stray eggs accidentally introduced. As the eggs of these species remain viable in the dry state for several months there is an ever present danger, when various cultures are being bred in the same laboratory, that eggs may adhere to instruments, glassware or the hands of the operator or fall onto bench surfaces and be accidentally picked up again. The whole position is very puzzling and must remain open for the time being. It is hoped to obtain further strains of both species and to repeat the experiments with these.

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References.

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EXPLANATION OF PLATE XIV.

Adult male showing combination of characters of *A. aegypti* and *A. albopictus*.